To the Corporation of

The Rockefeller Institute for Medical Research

New York City, N.Y.

Gentlemen:

The following is a report of the Scientific

work carried on in the wards and laboratories of the

hospital during the past year.

Report of Dr. Rivers with Dr. Valentine.

A. Clinical Study of Cases of Chicken-pox and Measles in
the Hospital. Fifty cases of chicken-pox were admitted to the iso-
lation ward for observation and treatment. In no instance was any
relationship between herpes zoster and chicken-pox noted.

Eleven cases of measles were admitted for treatment.

In the spring of 1926 during the outbreak of influenza,
fifteen cases of upper respiratory infection were admitted for
treatment.

B. Experimental Study of Chicken-pox in Monkeys. The
lesions observed in monkeys' testicles inoculated with emulsified
human varicella papules and vesicles were described in a previous
report. In the experiments reported at that time several species
of monkeys were employed as well as rats, rabbits, guinea pigs, and
chickens. As will be remembered, significant lesions, nuclear in-
clusions, were found only in the testicles of African vervets 5 and
6 days after inoculation, and not in other inoculated tissues of
the same animals, nor anywhere in the other experimental animals.
Furthermore, similar inclusions were not found in the testicles of
a vervet inoculated with normal skin. The inclusions were the
eosin-staining nuclear bodies which are consistently associated
with certain virus diseases and which, regardless of their nature,
indicate to many workers the presence of a virus. Therefore, in
view of these facts, it was deemed not unlikely that the acidophilic
nuclear inclusions in the vervets' testicles were manifestations
of the presence of a virus. The nature of the virus had not been
studied at the time of the previous report. Recently, however, ex-
periments were performed to obtain, if possible, additional inform-

ation concerning the suspected virus and the results of this work
will be presented briefly below.

In this work we were immediately confronted with the
problem of obtaining vervets. Green monkeys, a very closely re-
lated species, were available. These monkeys have also proven
satisfactory as nuclear inclusions have been found in their testi-
cles, 7 of 9, five days after injection of emulsified varicella
vesicles and papules.

The experiments reported at this time were conducted to
determine whether the eosin-staining nuclear inclusion in monkeys'
testicles inoculated with emulsified varicella lesions are speci-
fically associated with the virus of varicella. In connection with
this phase of the work six experiments, consisting of neutraliza-
tion and reinoculation tests, were performed. The results of the
work are summarized in the following table:

Summary of Results of Neutralization and Reinoculation Tests

Exp.	Monkey	State of Monkey	Inoculum	Nuclear Inclusions
1	76	Normal	Virus + convalescent serum	−
	82	"	" + normal serum	+
2	4	"	" + convalescent serum	−
	5	"	" + normal serum	−
3	62	"	" + convalescent serum	−
	68	"	" + normal serum	+
4	A	Actively immunized	Virus	−
	61	Normal	"	+
5	61	Actively immunized	Virus	−
	89	Normal	Virus + convalescent serum	−
	88	"	" + normal serum	+
6	63	Normal	Virus + convalescent serum	−
	80	"	" + normal serum	+

+ indicates presence of nuclear inclusions in testicles.
− indicates absence of nuclear inclusions in testicles.

Many viruses produce characteristic macrospic lesions in animals, or cause marked changes in their conduct. Such alterations and lesions serve as indications of virus activity. Under proper conditions the occurrence of those changes is specifically prevented either by mixing the virus with a homologous immune serum prior to the inoculation of the animal or by injecting the virus alone into an animal previously immunized by means of the same virus. In addition to the characteristic macroscopic lesions already mentioned, many viruses also produce equally characteristic microscopic changes as evidenced by the presence of inclusion bodies in the nuclei and cytoplasm of injured cells. Sometimes, however, the microscopic changes are the only manifestation of the presence of a virus. When such a condition arises, as it has in the work with varicella in monkeys, there is no obvious reason why the microscopic changes, inclusion bodies, should not be used as guides or indicators with the same degree of readiness as that with which the macroscopic lesions are employed.

Many workers believe that the eosin-staining nuclear inclusions are the manifestations of the presence of certain filterable viruses, amongst which is varicella. Consequently at the time of the previous report there were reasons for the belief that the nuclear inclusions found in the monkeys' testicles inoculated with emulsified varicella papules and vesicles were produced by the action of varicella virus. Proof of this was obtained by means of the experiments summarized above, and consists, in brief, of the following facts. Nuclear inclusions were not found in monkeys' testicles inoculated with a mixture of varicella virus and convalescent varicella serum. On the other hand, they were found in testicles inoculated with a mixture from varicella patients early in the disease. Furthermore, the in-

oculation of one testicle with varicella virus prevented the forma-
tion of nuclear inclusions in the other one when it was inoculated
at a later date with the same virus.

C. Experimental Study of Measles. Special interest in
measles has been provoked by the recent work of certain investiga-
tors. In view of this work, we felt that the study of measles
should again be undertaken here at the Institute. As yet enough
progress has not been made to warrant a detailed report.

D. Further Study of a Filterable Rabbit Virus (Virus III).
In previous reports a description was given of the growth and the
persistance of Virus III in a transplantable rabbit neoplasm. Since
then a study of the effect of Virus III upon the growth and malig-
nancy of the tumor has been made. This work was conducted with Dr.
Pearce and the results will be described by her.

Virus III, as will be remembered, is indigenous to rabbits
and was discovered in attempting to infect rabbits with the virus of
varicella. The rabbit virus offers an excellent opportunity for the
study of a virus disease in its natural host, a relatively inexpensive
laboratory animal. Since the virus in vitro is preserved with diffi-
culty, even in glycerine, it has been necessary to transfer it from
rabbit to rabbit at intervals of 4 days over a period of $3\frac{1}{2}$ years.
Such a procedure is time consuming and expensive. Nevertheless we
felt that the virus should be kept available. Recently, however,
with the aid of Dr. Hawkins, we have been able to show that the vi-
rus in testicles frozen and desiccated is active after a period of
3 months. Furthermore, the indications are that, under such condi-
tions, it will continue active for an indefinite length of time.

E. The Effect of Repeated Freezing (-185° C.) and Thawing
on Colon Bacilli, Virus III, Vaccine Virus, Herpes Virus, Bacterio-
phage, Complement and Trypsin. The effect of low temperatures on
bacteria, viruses, ferments, cells, and the bacteriophage has been
observed and reported. The effect of repeated freezing and thawing
on some of them has also been studied. The temperatures used, how-
ever, as well as the number of freezings and thawings employed, var-
ied greatly, and in many instances the effect on only one or two a-
gents was studied by the same worker. Nevertheless some observers
have stated that by means of repeated freezing and thawing it is
possible to determine whether certain active agents are living or
dead. In view of the inadequacy of the knowledge concerning this
subject it seemed desirable to study further the effect of repeat-
ed freezing (-185° C.) and thawing on vaccine virus, Virus III,
herpes virus, and the bacteriophage as compared with that on colon
bacilli, complement, and trypsin.

The low temperature (-185° C.) used in the work describ-
ed below was obtained by means of commercial liquid air which was
transported from the plant to the laboratory in Dewar flasks. De-
sired amounts of the air were transferred to deep Dewar beakers
where small amounts of the substances to be frozen, enclosed in
Noguchi tubes, were completely immersed for several minutes. Af-
ter the substances had been completely frozen, they were quickly
thawed in tap water (16° to 18° C.). This procedure was repeated
as often as desired. Then the treated substances, as well as the
controls, were tested and titrated by standard methods.

All experiments made to determine the effect of freez-
ing (-185° C.) and thawing on colon bacilli, Virus III, vaccine

virus, bacteriophage, complement, and trypsin were repeated several times and many of them were performed simultaneously. For convenience, however, the results of the experiments will be reported separately.

Colon Bacilli. It is a well established fact that many bacteria are killed by repeated freezing and thawing, even when a temperature only a few degrees below 0° C. is employed. Colon bacilli are no exception to the rule. We were particularly interested, however, in determining what influence the number of bacteria per cc. and the type of suspending fluid have on the percentage of organisms killed by repeated freezing and thawing. Our experiments indicate that colon bacilli are more susceptible to repeated freezing and thawing when Locke's solution instead of broth is employed as a suspending fluid, that approximately the same percentage of bacteria is killed in the different suspensions made by means of broth, and that a greater percentage of bacteria is killed in the 1-1,000 dilution made with Locke's solution than in the 1-10 dilution made with the same fluid.

Virus III. Virus III is easily killed or inactivated by repeated freezing and thawing in spite of the high protein content of the emulsion containing the virus.

Vaccine Virus. A certain percentage of vaccine virus is dead or inactive after 34 successive freezings and thawings, provided the emulsion containing the virus has been diluted 1-1000 with Locke's solution before it is frozen and thawed. This virus, however, proved to be more resistant than any other of the active agents tested.

Herpes Virus. Herpes virus, provided the brain emulsion

containing it has been sufficiently diluted by means of Locke's solution, is killed or inactivated by repeated freezing and thawing.

Colon Bacteriophage. The results of our experiments afford conclusive evidence (1) that phage is inactivated by repeated freezing (-185° C.) and thawing and (2) that the type of diluent influences the percentage of phage inactivated in this manner. Furthermore, it has been shown that an increase in the dilution of the stock filtrate of phage by means of broth and distilled water does not at the same time lead to an increase in the percentage of phage inactivated by repeated freezing and thawing. On the other hand, an increase in the dilution accomplished by means of physiological salt solution and Locke's solution does lead to an increase in the percentage of phage inactivated, as evidenced by a greater percentage of phage being inactivated in dilutions of 1-1,000 than in dilutions of 1-10. These findings are similar to the ones obtained concerning the effect of dilution on the percentage of bacteria killed by repeated freezing and thawing.

Complement. The titer of complement in undiluted serum and in serum diluted 1-10 is not appreciably decreased by 12 successive freezings and thawings. Complement, however, in serum diluted 1-50 and 1-100 is inactivated by such treatment.

Trypsin. Portions of a 5 per cent stock solution of trypsin were diluted 1-10 and 1-500 by means of a buffered solution having a pH of 7. Half of each specimen was used as a control, the other half was frozen (-185° C.) and thawed 12 times. Care was taken to inactivate none of the ferment by

shaking. After the amounts of active trypsin in the control and treated specimens were determined and compared it was found that 70 per cent of the trypsin was inactivated in the specimen diluted 1-500 while only 17 per cent was inactivated in the one diluted 1-10. These results afford conclusive evidence that trypsin is inactivated by repeated freezing and thawing and that under these conditions a greater percentage is inactivated in a dilute solution than in a concentrated one.

Dr. Northrop furnished the trypsin and determined the amount of active trypsin in the control and treated specimens.

The numerous discussions concerning the nature of the bacteriophage have led many investigators to question more closely the living nature of some of the so-called filterable viruses. Many tests have been devised to act as criteria for the presence of life, but so far no one of them has been found satisfactory. Recently, Sanderson, using a temperature of -78° C., found no diminution in the titer of two strains of phage subjected to 20 successive freezings and thawings. Since bacteria and cells are killed by repeated freezing and thawing, he concluded that the bacteriophage must be something other than a living organism. In the experiments described in the present report, a number of active agents, some undoubtedly alive, others equally unquestionably not alive, and still others of a doubtful nature, were subjected to repeated freezing (-185° C.) and thawing. In this way it has been possible to show, as evidenced by the results of the experiments, that the mere destruction or the inactivation of a substance by such treatment is not proof that it is possessed of life.

F. Study of Certain Gram-negative Bacilli Found in Cultures from Throats.

For several years before coming to the Institute I worked with the hemophilic group of bacteria and along with Dr. Fildes and Dr. Avery I showed that influenza bacilli (Pfeiffer) require for successful cultivation two accessory growth factors, one autoclave labile (v), the other autoclave stable (x). In addition to this, I found that, by means of these two factors, the Gram-negative hemophilic bacilli occurring in cultures from different sources can be grouped according to their growth requirements, - some require the addition of only one factor, others require the addition of both. The following table shows which factor must be added in order to successfully cultivate the different so-called hemophilic bacteria.

Type Bacillus	Source	V Factor	X Factor
Portussis bacilli	Human	-	-
Influenza bacilli	"	+	+
Parainfluenza bacilli	Human and Cat	+	-
B. hemoglobinophilus canis	Dog	-	+
Hemolytic influenza bacilli	Human	+	+

+ indicates factor must be added
- indicates factor need not be added.

The findings concerning the growth requirements of the different hemophilic bacilli have been confirmed and extended by other workers. It has been shown that the Koch-Weeks bacillus requires both x and v for growth and that it is identical with the influenza bacillus (Pfeiffer). Most workers, however, found that the hemo-

lytic influenza bacilli, first described by Pritchett and Stillman, require the addition of only the v factor. Fildes, on the other hand, has recently reported that some of the hemolytic influenza bacilli require both x and v factors while others require the addition of only the v.

Numerous throat cultures and interest in the problem on the part of Dr. Valentine offered the opportunity of resuming the work on the group of hemophilic bacilli. The following table shows that, from 45 throats cultured, 17 strains of non-hemolytic influenza bacilli and 15 strains of hemolytic influenza bacilli were obtained. Of the non-hemolytic strains, 15 required for growth the addition of v and x, and 2 required the addition of only v. Of the 15 hemolytic strains, 10 required the v factor alone, 3 required both the v and x factors, and 2 would grow through only 2 transplant generations in broth to which both v and x had been added. The 10 strains that required the addition of only the v factor grew rapidly on rabbits' blood agar and produced wide zones of hemolysis around the colonies. The remaining 5 strains formed small colonies with narrow zones of hemolysis.

Occurrence of Hemophilic Bacilli.

Disease	No. throats cultured	Non-hemolytic bacilli	Hemolytic bacilli
Chicken-pox	27	8	7
Measles	9	5	3
Influenza ?	4	1	4
Mumps	1	1	0
Miscellaneous	4	2	1
Total	45	17	15

Since certain strains, as indicated above, require for growth the addition of only the v factor while others require the addition of only the x factor, it seemed of interest to determine whether members of these two groups will continue to grow symbiotically through an indefinite number of transplant generations in broth to which neither of the growth accessory factors has been added. This was accomplished repeatedly in the following manner.

A = **B. hemoglobinophilus canis,** requires addition of x factor.

L = **B. parainfluenza,** non-hemolytic, requires addition of v factor.

B = Hemolytic influenza bacillus, requires addition of v factor.

The media employed was autoclaved meat infusion broth, 0.5 cc. used in making transfers.

A	4th generation - no growth as shown by test on blood agar
L	3rd " " " " " " " " " "
B	3rd " " " " " " " " " "

A + L 7th generation - good growth. Subculture on blood agar plate showed 2 type colonies. From these A and L were recovered.

A + B 7th generation - good growth. Subcultures on blood agar plates showed hemolytic and non-hemolytic colonies. From these A and B were recovered.

These experiments make it seem not unlikely that the bacilli requiring the addition of only the x factor synthesize the v, and that the ones requiring the addition of only the v factor synthesize the x. That this is what actually occurs has become more evident through other experiments the details of which need not be given at the present time.

Publications.

Rivers, T.M. Nuclear Inclusions in the Testicles of Monkeys Inject-
ed with the Tissue of Human Varicella Lesions.
J. Exp. Med. 1926, XLIII, 275.

Varicella in Monkeys.
Nuclear Inclusions Produced by Varicella in the
Testicles of Monkeys. (In press)

Effect of Repeated Freezing (-185° C.) and Thawing
on Colon Bacilli, Virus III, Vaccine Virus, Herpes
Virus, Bacteriophage, Complement, and Trypsin.
(In press).

Studies Concerning Acute Respiratory Diseases.

Report of Drs. Avery and Heidelberger with Dr. Goebel.

I. Chemistry of the Soluble Specific Substance.

A. Type III pneumococcus. Since the properties of the specifically reacting polysaccharide of Pneumococcus mucosus had previously been established it was considered proper to add glucose to the culture medium. Greatly increased growth was thus induced, and the yields of soluble specific substance rose from 6-9 gm. per 300 liters to 35-40 gm. With these increased amounts of material, identical with the soluble substance as previously obtained without the aid of glucose, it has been possible to throw new light on the structure of the specific polysaccharide.

When hydrolyzed in the cold with 75 per cent sulfuric acid the Type III substance yields a variety of acid products which it is possible to separate. These acids probably vary in molecular weight, but all show one carboxyl group for every two sugar nuclei, as the acid equivalents range between 310 and 343. No glucose could be detected. On further hydrolysis by boiling for 5 hours with normal sulfuric acid the specific substance yields 9.5 per cent of glucose and 85 per cent of a disaccharide acid differing from any known non-nitrogenous sugar derivative. This acid is precipitated by basic lead acetate and purified by conversion into a crystalline morphine salt which melts at 153-6°. The sugar acid, recovered from the salt, is a white, amorphous powder with $[\alpha]_D$ + 10.0°, a reducing power of 49.5 per cent, calculated as glucose, and an acid equivalent of 363 as against 356 calculated for $C_{11}H_{19}O_{10} \cdot COOH$. The acid gives the naphthoresorcin test characteristic of sugar acids of the glucuronic type. Except for a slight difference in $[\alpha]_D$, which was +7.8°, the crude

disaccharide acid corresponded very closely with the purified material in its properties. The reducing group of the disaccharide acid reacts quantitavely by the method of Willstätter and Schudel, thus showing it to be aldehydic in function, and the substance may therefore be termed an aldobionic acid. When boiled for 20 hours with normal sulfuric acid much of the aldobionic acid was recovered unchanged. The filtrate from this contained 20 per cent of the starting material present as reducing sugars, calculated as glucose, gave $[\alpha]_D$ +54.1°, and readily yielded glucosazone melting at 203-4°, $[\alpha]_{D'}$ initial, -54.5°. final, -30° in pyridine - alcohol. Thus glucose appears as a product of further hydrolysis of the aldobionic acid, while the acid half of the molecule is apparently destroyed by the prolonged boiling with acid. Potassium acid saccharate was isolated after oxidation of the aldobionic acid with strong nitric acid. No mucic acid could be found, so that the acid half of the molecule can scarcely be galacturonic acid. On oxidation with bromine the disaccharide acid yields a dicarboxylic acid, but this is as difficult to hydrolyze further as is the aldobionic acid itself.

The above data are believed to show that the disaccharide acid is formed by condensation of glucose and a hexose-uronic acid, not galacturonic acid, in such a way that one reducing group and the carboxyl remain free. It is thus a nitrogen-free analog of chondrosin, the nitrogen-containing disaccharide acid found in chondroitin sulfuric acid, a component of the mucroproteins.

Since no glucose is split off during the preliminary hydrolysis of the Type III specific polysaccharide with 75 per cent sulfuric acid, and this sugar is formed in small amount on long-continued hydrolysis of the aldobionic acid, it is believed that the glucose

liberated during the hydrolysis of the polysaccharide owes its origin, not to a separate part of the carbohydrate molecule, but chiefly to the secondary reaction involving the aldobionic acid. Since, also the initial partial hydrolysis products show one carboxyl group for each two sugar nuclei, it would seem that the polysaccharide as a whole is built up of units of the aldobionic acid. A substance of this composition, $(C_{12}H_{18}O_{11})_n$, should have an acid equivalent of 338 and a carbon and hydrogen content of 42.6 and 5.4 per cent, respectively. A comparison of these figures with the experimental values shows an almost exact correspondence.

It is therefore believed that the specific polysaccharide of the Type III penumococcus is a definite chemical individual built up of units of a difficulty hydrolyzable disaccharide acid in which glucose and a hexoseuronic acid are combined in such a way that one aldehydic group and the carboxyl remain free. The polysaccharide is thus unusual not only in its possession of immunological specificity but in its chemical constitution as well. Whether its unusual structure bears any relation to its immunological properties must be left for future work to determine.

B. Type A Friedlander Bacillus. By methods essentially the same as those used in the case of the soluble specific substances of Pneumococcus and the Type B Friedlander bacillus a nitrogen-free polysaccharide with specific properties was isolated from the Type A Friedlander bacillus. It is a white, amorphous powder with marked acidic properties, and is readily soluble in water and dilute alkali. It rotates the plane of polarized light about 100° to the left, and on hydrolysis yields glucose and a sugar acid, recalling the behavior of the soluble specific substance of Type III pneumococcus. A further

study of the Friedlander polysaccharide, especially of the acid
fraction yielded on hydrolysis, is in progress.

C. A Soluble Specific Substance derived from Gum Arabic.
The wide distribution of specifically reacting polysaccharides among
microorganisms of the most diverse types made it seem not impossible
that such carbohydrates might be found among products derived from
the higher plants. A test of a number of water-soluble gums of plant
origin was accordingly made, and occasional samples of gum arabic
(gum acacia) were found which precipitated Type II (and Type III) anti-
pneumococcus serum at as high adilution as 1:25,000, but did not pre-
cipitate Type I antiserum or normal horse serum. By fractional hydro-
lysis with 1:1 hydrochloric acid the specific activity of the recover-
ed gum was increased 100 - 150 fold, about one-half of the pentose
present being removed. This method of purification did not consist
merely in hydrolysis of accompanying inert carbohydrates, since up to
50 per cent of the original gum was recovered in the highly active
fraction. It is therefore possible that gum arabic is converted into
a specific polysaccharide by removal of certain pentose groupings in
glucosidic union.

After further purification the specific fraction forms a
white powder containing 0.08 per cent of nitrogen. It is readily
soluble in water and has marked acidic properties. $[\alpha]_D$ of the
free acid is about -10° and that of the sodium salt about -14°. The
gum gives the naphthoresorcin test characteristic of substances con-
taining acids of the glucuronic type. At a dilution of 1:5,000,000
it still reacts with Type II antipneumococcus serum, and it also
precipitates Type III antiserum, although not at such high dilutions.
On hydrolysis with boiling normal sulfuric acid the specific gum

readily yields crystalline galactose and at least two complex sugar acids. One of these corresponds roughly to a disaccharide acid, with an acid equivalent of 427, a reducing power of 44.6 per cent, and $[\alpha]_D$ of +8.8°, recalling the analoguus aldobionic acid shown to be the chief hydrolysis product of the soluble specific substance of Type III pneumococcus, and the acid hydrolysis product of the specific polysaccharide derived from the Type A Friedlander bacillus. If these preliminary findings are borne out by further work it would seem that disaccharide acids play an important role in the structure of many polysaccharides of specific function. The specific gum arabic is particularly well suited as material for a chemical study of acids of this type, since it may be obtained in unlimited amount.

Precipitation of the specific gum arabic fraction with Type II pneumococcus antibody solution and recovery of the polysaccharide from the immune precipitate gave a product resembling in its properties the material taken, but with an enhanced reactivity not only for Type II antiserum, but for Type III antiserum as well. Had the substance in the specific gum capable of reacting with Type III serum not been the same as the portion reacting with the Type II serum, it should have been left behind by a specific precipitation with Type II antibodies. It would therefore appear that the specific gum arabic contains in a single substance the molecular groupings necessary for interation with antibodies in both sera. The greater variety of the hydrolysis products of the specific gum than in the case of the specific polysaccharides of bacterial origin hitherto studied is also in agreement with this view.

The reactivity of the specific gum arabic with Type II

and Type III antipneumococcus sera recalls the relationship of the
soluble specific substance of Type B Friedlander bacillus to that of
Type II pneumococcus, and may possibly also be accounted for on the
basis of heterogenetic specificity.

The relationship has already been alluded to between the
specific polysaccharide of Type III pneumococcus and the carbohyd-
rate portion of the mucoproteins. There is as well as a certain
analogy to the pectins discernible in several of the substances
studied. In this connection the finding of a specifically react-
ing polysaccharide as a constituent of gum arabic is of interest,
and it is not impossible that sugar derivatives with specific pro-
perties may occupy a position of biological significance among
higher forms of life as well as among bacteria.

II. Purification of Pneumococcus Antibodies.

Felton's method, modified in a few details and made more
precise, was adopted as the most convenient method for the rapid
concentration of pneumococcus antibodies, and the solutions so ob-
tained were used as starting material for further purification.
The fractionations were controlled by agglutination tests and oc-
casional protection tests.

Of all the protein precipitants tested picric acid ap-
peared to offer most promise. Type I antibodies were generally
precipitable by this reagent before all protein in the solution
came down, while in the case of Type II antibody solution a small,
relatively inert protein fraction could usually be precipitated
before the main portion of the antibodies was thrown down. However,
inactivation of the antibodies frequently occurred, and as this was
believed to be due to the acidity of the picric acid, less strongly

acid derivatives of this substance were tried; namely, picramic acid and 2.6- dinitrophenol. No fractionation could be observed with picramic acid.

When a Type I antibody solution was added to 2 volumes of saturated aqueous dinitrophenol (DNP) a relatively inactive fraction containing about 18 per cent of the protein was thrown down. With larger amounts of DNP solution no precipitate was obtained. In the case of Type II antibodies a first fraction similar to that obtained with the Type I solution was thrown down when 4 volumes of DNP solution were used. On cautious addition of dilute sodium hydroxide to the supernatant a point could generally be reached at which most of the agglutinins were precipitated, although occasionally some antibodies remained in the supernatant. The use of DNP finally discarded owing to the difficulty of removing it without loss of antibodies and because of the relatively slight degree of purification obtainable by its aid.

A large number of adsorption experiments were then tried, using highly active, specially prepared alumina and purified kaolin. Concentration, p_H, and amount of adsorbent were varied in many ways, and while it often seemed that relatively much protein was being adsorbed and relatively much antibody left behind the figures obtained were not sufficiently far apart to give the impression that any far-reaching purification had been effected. The difficulty of interpreting protection and agglutination tests on any quantitative basis rendered caution necessary in ascribing real significance to any results other than those showing an extensive separation of protein and antibody. Such results were seldom obtained in the course of this work, and generally failed of repetition.

Almost all of the antibody solutions showed an optical rotation of between -42° and -49°, but the fractions obtained from the same solution varied little, nor were there significant differences between the Type I and Type II solutions.

Experiments on the electrodialysis of antibody solutions were also carried out, using various membranes and types of apparatus, but no encouraging degree of fractionation was observed.

Dr. Avery and Dr. Tillett.

Studies on Pneumococcus protein Immunity.

In the immunological mechanism of the pneumococcus two separate and distinct constituents of the cell have been found to be operative: - one, the carbohydrate; the other, the protein of the organism. The antigenic function of these two substances give rise under certain conditions to two distinct antibodies in immune serum. One, the anti-carbohydrate antibody which is type-specific and which affords specific protection against infection in animals. The other is the anti-protein antibody which is species specific, but which does not react with the soluble type-specific substance (carbohydrate) of the cell nor can afford passive protection against the virulent microorganism. This antibody exhibits only the broader protein-anti-protein reactions of the species, precipitating solutions of the protein of all types, and agglutinating only the non-encapsulated degraded "R" strains of Pneumococcus.

The knowledge that the protein constituent of the cell, under certain conditions, gives rise in the animal body to an immune substance which differs in its immunological reactions from the type-specific antibodies, has led to an inquiry as to the occurrence and

significance of this antibody in human serum. The anti-protein anti-body has been demonstrated in the blood serum of normal individuals in whom there is no history of a previous attack of pneumonia. While the number of observations are at present limited, studies are now in progress to determine the frequency of occurrence and percentage incidence of this antibody in the blood of healthy persons. Moreover, the same immune body has been demonstrated in the serum of patients during the course of lobar pneumonia. Unlike the type-specific (anti-carbohydrate) antibody, which appears in the blood at or about the time of crisis, the anti-protein substance has been found in significant amounts as early as forty-eight hours after the onset of the disease.

While as yet the observations are but few in number, these positive findings reveal in human serum the presence of a hitherto unrecognized antibody which is specifically related to the antigenic protein of Pneumococcus. The significance of the occurrence of this antibody in human sera, and its possible relationship to natural immunity in man, and to the immunological processes of recovery in disease await further investigation.

Dr. Tillett.

Studies on Immunity to Type III Pneumococcus.

In the preceding report certain peculiarities of the reaction of the rabbit to Type III pneumococcus infection were brought out. These were:

1. Low virulence of this organism for rabbits.

2. Absence of type specific antibodies in the blood serum of rabbits immunized with Type III Pneumococcus.

3. Presence of anti-protein antibodies which are species